THE NATURAL HISTORY OF HUMAN POLIOMYELITIS

I. Distribution of Virus in Nervous and Non-Nervous Tissues*

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The distribution of virus in the tissues of an infected host is frequently, if not always, a key to the nature of the disease, and experimental studies with a number of neurotropic viruses in recent years have shown that a proper investigation of such distribution can point not only to the site from which the virus invades the nervous system but also to its mode of spread and elimination.

Our present concepts of the nature of human poliomyelitis are based (*a*) on certain limited investigations of human tissues made for the most part 25 to 30 years ago when the virus was first recognized and the criteria for its identification were not always rigid, and (*b*) on the behavior of the virus in experimental animals, different species of which appear to behave quite differently (1, 2). The scattered existing data on the infectivity of human tissues include tests on the spinal cord, medulla, and cerebrospinal fluid, the lymph nodes including the tonsils and pharyngeal tissue, the nasal mucosa, the blood and viscera, and as regards secretions and excretions, nasopharyngeal washings and intestinal contents or stools. No virus has been demonstrated in the cerebrospinal fluid, the blood, or the viscera. Earlier reports of the presence of virus in the lymph nodes, particularly those of the mesentery, could not be corroborated by numerous subsequent tests and the explanation was offered that in the early work the lymph nodes might have been contaminated by admixture with positive tissues (3). Recently, however, the occasional presence of virus in human cervical and mesenteric lymph nodes has again been reported, although the criteria used for identifying the virus in some instances are open to question (4, 5). The early repeated positive results with the tonsils and attached pharyngeal mucosa (6–8) have not been reinvestigated in recent years and it has re-

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mained a question whether the virus was present in the tonsils or in the pharyngeal mucosa. The nasal mucosa has been tested in only three cases and the virus was reported to be present in one (8). The numerous tests with nasopharyngeal washings through the years have yielded a certain number of positive results, although it has never been made clear whether the virus came from the nose or from the mouth and pharynx. The infectivity of the intestinal contents which had been denied for many years was established beyond doubt in more recent work, although the source of the virus thus eliminated remained unknown. In no instance, however, have all the tissues and secretions or excretions upon which tests are recorded been studied simultaneously in the same case. It was clear, therefore, that the existing data, even including the controversial studies, were insufficient for formulating any idea of the essential nature of human poliomyelitis.

Plan of Study

The plan of the present sudy was to search for the virus of poliomyelitis in a sufficiently large number of properly selected tissues to enable one to determine whether or not its distribution follows a distinct pattern. Although past experience indicated that the primary transmission of the virus from human tissues to monkeys is not always readily accomplished, it nevertheless appeared possible that if all the selected tissues were obtained from each of six or more cases of human poliomyelitis and tested in the same manner, each specific structure would be inoculated into monkeys a sufficient number of times to give at least an indication of the presence or absence of virus in it. The selection of tissues for these tests was determined by their capacity to indicate (*a*) whether or not a certain system was affected, (*b*) what might be the centrifugal or centripetal pathways pursued by the virus outside the central nervous system, and (*c*) whether at the time of death the virus is distributed indiscriminately throughout the central nervous system or is present in appreciable amounts in some areas and not in others in accord with a definite pattern.

Table I lists the tissues selected for study and the order in which they were removed from the body. The pharyngeal mucosa with or without the tonsils was tested not only because in the work of 25 to 30 years ago the virus was reported most regularly as being present in the tonsils, but also because the pharynx is part of the alimentary tract and the tonsils are part of the cervical lymphatics. Practically all the superficial and deep cervical lymph nodes, and the mesenteric lymph nodes were dissected out and tested because the presence or absence of virus in them is still a matter of controversy. The axillary and inguinal lymph nodes were included for control in order to determine whether the virus may possibly be widely distributed in the lymphatic system and be responsible for the pathological changes which are so frequently encountered in it. The liver, spleen, kidneys, and lungs were selected as an index to the generalization of the virus, but we now think that it was a mistake to include the lungs in the pool because of the frequency of aspiration of material from the alimentary tract in terminal cases. The

suprarenals were tested chiefly because they contain so many neurons of the autonomic system which might be involved in case appreciable centrifugal spread of virus occurred. The entire submaxillary and part of the parotid salivary glands were obtained to determine whether or not the virus of poliomyelitis, like that of rabies, may spread to them centrifugally, and whether or not the saliva could thus become one means of elimination. 12 to 18 inches of ileum, near the ileocecal junction, and of descending colon with their respective contents were tied off and excised. The intestinal contents and the thoroughly washed walls were tested separately. The presence or absence of virus in the wall of the gut with its nerve cells of the submucous and myenteric parasympathetic plexuses (Meissner's and Auerbach's) would throw considerable light not only on the origin of virus in the stools, but taken together with the other data, also on the possible site or sites from which the nervous system may be invaded. The cervical and abdominal sympathetic ganglia were tested to indicate whether or not centripetal or centrifugal spread of virus occurs along their course. The nasal mucosa, both the nervous, olfactory, and the respiratory portions, was included to determine whether or not it is a site

TABLE I

Human Poliomyelitis Necropsies

Tissues Selected for Study and Order of Their Removal

1. Pharyngeal mucosa with or without tonsils
2. Axillary and inguinal lymph nodes
3. Suprarenals
4. Abdominal sympathetic ganglia
5. Liver, spleen, kidneys, lungs
6. Mesenteric lymph nodes
7. Ileum (near ileocecal junction) and contents
8. Descending colon and contents
9. Cervical lymph nodes
10. Salivary glands (submaxillary and parotid)
11. Superior cervical sympathetic ganglia
12. Olfactory bulbs
13. Anterior perforated substance and adjacent corpus striatum
14. Diencephalon
15. Pons and medulla
16. Anterior frontal and occipital portions of neopallial cortex
17. Motor cortex
18. Mesencephalon
19. Spinal cord
20. Nasal mucosa (olfactory and respiratory)

from which infective virus may be eliminated. In the central nervous system, tests on the olfactory bulbs and anterior perforated substance were expected to throw light on whether or not the virus invades by the olfactory pathway. This deduction, however, would be possible only if it were found that there was no generalized diffusion of the virus involving areas such as the anterior frontal and occipital portions of the neopallial cortex in which no neuronal lesions are found. The diencephalon, mesencephalon, motor cortex, pons and medulla, and spinal cord were tested to check the frequency with which the virus may be isolated from regions in which the lesions are usually localized.

Since the olfactory pathway has received special consideration as a possible portal of entry of the virus and since the pathological changes observed in the olfactory bulbs of monkeys infected by the nasal route (9) were not found in human olfactory bulbs (10), it was especially desirable to establish whether or not they contained the virus. Consequently, it was planned to study several additional cases in which only the olfactory bulbs and spinal cord would be investigated.

Methods

In preceding studies on human poliomyelitis tissues have usually been sent to the laboratory (occasionally all mixed together in a single bottle) by pathologists unaware

of the precautions that must be observed. Our material was derived from necropsies carried out by ourselves under relatively aseptic conditions and with a sufficient number of sterile instruments to permit the individual handling of each tissue or group of tissues to be tested. The necropsies were performed in Indiana, Ohio, and West Virginia where a moderate epidemic of poliomyelitis occurred in 1940. In some instances it was possible to begin the necropsy within a few hours after death while in others longer delays were unavoidable.

The skin was prepared with iodine and alcohol. The usual incision was used for obtaining the thoracic and abdominal tissues. To expose the salivary glands and other structures of the neck, the original incision ending on the shoulder over the acromion process was carried backwards along the upper edge of the scapula which permitted reflection of the skin of the neck up to the mandible. To obtain the abdominal sympathetic ganglia, the descending aorta was exposed at the level of the left suprarenal and the large celiac ganglia and associated plexuses were dissected; a small piece was occasionally sectioned for histological confirmation of the tissue. No attempt was made to expose the skull aseptically. However, after the scalp had been reflected, the area was washed with alcohol and the bone, through which the saw was to pass, was cleared of periosteum and similarly treated with alcohol. A sterile saw was used but great care was taken not to incise the dura. After removal of the calvarium, all further work was done with sterile rubber gloves since the brain had to be removed manually. In order to avoid tearing or maceration of the olfactory bulbs, the frontal poles were lifted with a sterile spatula and the olfactory tracts were cut before the brain was removed from the cranial cavity. The brain was placed on sterile towels and the various portions, dissected out with separate instruments, were put into individual bottles. The nasal mucosa was obtained last through an intracranial approach after stripping the dura mater and thorough cleansing with alcohol of the bones forming the roof of the nose. The order in which the tissues were removed, as indicated in Table I, was arrived at after some trial and error and was chosen chiefly because it permitted the dissection of the various structures in relatively bloodless fields.

In the beginning, the tissues were kept in 50 per cent, buffered glycerol, transported to the laboratory in the frozen state packed in solid CO_2, and stored in the refrigerator at about 5°C. until used. Later on, however, the tissues (without glycerol) were frozen immediately after removal from the body and kept frozen with solid CO_2 until tested. Although we now prefer the latter method, there was actually no difference in the results obtained with the tissues preserved by either procedure. Representative sections of the nervous system (usually cervical, thoracic, and lumbar levels of the spinal cord and one level of the medulla to include the nuclei of the 9th and 10th cranial nerves) were obtained in each case for histological study to ascertain whether or not the pathological findings were in accord with the clinical diagnosis of poliomyelitis.

Preparation of Tissues for Inoculation.—With the exception of material such as nasal mucosa, pharyngeal wall, or tonsils, ileum, colon, and their respective contents, which are naturally contaminated, all the other tissues were prepared for inoculation as heavy suspensions in distilled water or physiologic salt solution, and the aseptic precautions which were observed in obtaining them were sufficiently adequate to prevent death of the animals from bacterial infection. Both olfactory bulbs were ground without added abrasive and the total amount, usually 1.5 to 2 cc. was injected intracerebrally (i.c.). With the exception of the spinal cord and medulla approximately 5 gm. of each of the

other parts of the nervous system were used, unless, as in the case of the diencephalon or anterior perforated substance, the entire specimen weighed less than 5 gm. Whenever possible the nervous tissues were ground without abrasives, and the total milky suspensions after filtration through several layers of gauze were inoculated i.c. and intra-abdominally (i.p.). The lymph nodes and salivary glands which frequently exceeded 10 to 15 gm. were used *in toto* and were inoculated as slightly centrifuged 20 per cent suspensions. Approximately 10 gm. of the pool of viscera were used for preparing a 20 per cent suspension, all of which was inoculated into a single monkey. The contaminated tissues were prepared differently. The nasal mucosa and the pharyngeal mucosa with or without the tonsils were ground with sand and taken up in sufficient distilled water or saline to yield a 10 per cent suspension. After horizontal centrifugation at about 2000 R.P.M. for 10 minutes, the supernatant liquids were drawn off, mixed with 15 per cent of their volume of anaesthetic ether, shaken for 10 minutes, and left in the refrigerator overnight. This was followed by horizontal centrifugation at 2000 R.P.M. for 10 to 20 minutes, and the middle layer between the sediment and the fatty cake at the top was pipetted off and again centrifuged at the same speed for 30 to 60 minutes. The final supernatant liquid was almost always sterile on blood agar, and was used for i.c. and i.p. inoculation. The original sediment before ether treatment was occasionally resuspended and used for nasal instillation in the same monkey which received the ether-treated material. The loops of intestine were opened in a large Petri dish and the contents separated from the wall with the aid of wooden tongue-depressors. The wall was then cut in several pieces, shaken several times with fresh changes of 500 to 1000 cc. of tap water, and left to wash in running tap water until the wash water was quite clear; the water was drained off and the intestinal wall was ready for use. As a rule, approximately 10 gm. of the intestinal wall were ground with sand and enough distilled water or saline to make a 20 per cent suspension. This suspension was then submitted to the same ether treatment and series of centrifugations which were described for the nasal mucosa and pharyngeal wall. With the exception of the ileum and colon contents, all tissues were inoculated i.c. and, whenever more than 4 cc. of suspension was available, i.p. as well. The ileum and colon contents were weighed and, depending on the amount, 10 or 20 per cent suspensions were prepared, and the same procedure of etherization and centrifugation, described for the other contaminated tissues, was followed here. On several occasions it was necessary to repeat the process of etherization and centrifugation two or three times before it was possible to obtain material that would not kill the inoculated monkeys within 24 to 48 hours. Since specimens so treated finally produced typical poliomyelitis in monkeys, the procedure is obviously not too harmful for the virus. The sediments from the first centrifugation before etherization were resuspended and used for nasal instillation (repeated daily for 6 to 10 days) in the same monkeys which received the ether-treated material i.p. and occasionally i.c. Whenever enough material was available two i.p. inoculations of 20 cc. each were given 24 or 48 hours apart.

Animals and Observations.—Since *Macacus rhesus* monkeys were the only ones available in sufficient numbers for this study, they were used in most of the tests. *Macacus cynomolgus* monkeys have been reported by several observers as being somewhat more susceptible. They were, therefore, used for a series of tissues from two cases, although in a comparative test with the spinal cords from five human cases which failed to produce poliomyelitis in *rhesus* monkeys on first inoculation, the *cynomolgi* did not prove to be superior in revealing the virus. Intracerebral inoculations were made under local anes-

thesia with novocain and adrenalin, because it was possible to give nasal instillations of contaminated material to such monkeys without deaths from pneumonia which occurred with great frequency among the animals that had had ether anesthesia. Rectal temperatures were taken daily and the animals were exercised to permit the detection of even slight degrees of paralysis as reflected in abnormal running or climbing. If the animals remained well during the first week or two they were frequently given a second intracerebral inoculation of the same material preserved in the refrigerator, and if they still developed no suggestive signs they were kept for 5 to 6 weeks after the first inoculation and were then killed for histological study. As a rule, 6 levels of spinal cord (3 lumbar, 1 thoracic, and 2 cervical) and one level of medulla (through the 4th ventricle) were sectioned, although in certain instances other regions of the nervous system were also examined. The olfactory bulbs and anterior perforated substance were included whenever nasal instillations had been given. Care was taken to include the roots in the sections of the spinal cord, because their condition can be of great assistance in establishing a diagnosis of non-paralytic poliomyelitis (11). The remaining portions of the spinal cord and medulla were saved in 50 per cent buffered glycerol and passage was often resorted to when the diagnosis was in doubt. The viscera were examined in every monkey and it is noteworthy that only one case of tuberculosis was encountered among the more than 200 animals used in this study. The human spinal cord or medulla and pons suspensions were also injected i.c. and i.p. into 6 to 10 young mice. The mice, however, remained well and had no immunity 2 months later to Armstrong's mouse passage virus (Lansing strain).

Criteria for Diagnosis of Experimental Poliomyelitis.—When a monkey developed unmistakable flaccid paralysis of one or more extremities with characteristic neuronal necrosis, neuronophagia, and infiltrative lesions in the spinal cord, the diagnosis of experimental poliomyelitis was made without resorting to passage in each instance. Positive passage was, however, obtained with one or more tissues in most cases, which taken together with the non-pathogenicity of the material for mice, was considered sufficient basis for diagnosing the presence of poliomyelitis virus in the human material. In the preceding communication (11) we described the occurrence of non-paralytic poliomyelitis, established by histological examination, with typical lesions in the spinal cord in monkeys inoculated with human virus. Although the diagnosis of non-paralytic poliomyelitis was made eleven times, it was not necessary to base any significant conclusion on that diagnosis alone, since in all but one instance, either the same tissue from other cases gave rise to typical paralysis or positive passage with the development of typical paralytic poliomyelitis was obtained. Histologically the diagnosis of experimental poliomyelitis was not made without evidence of past or present neuronal lesions in the spinal cord, despite the one exception which we have encountered (11).

RESULTS

Eleven complete necropsies were performed in which all the tissues enumerated in Table I were obtained. Two of these turned out not to be poliomyelitis on histological examination of the human spinal cord and medulla and the monkeys inoculated with these tissues remained well. It is of interest to note with respect to the type of disease that may be clinically confused with poliomyelitis, that one of these cases was most likely

an osteomyelitis of the leg with a terminal pyogenic pleuritis, pneumonia, pericarditis, and focal myocardial abscesses, and the other was an infectious polyneuritis (neuronitis) with typical lesions in the nerve roots. In two additional cases of poliomyelitis only a limited number of tissues were obtained.

To permit a critical analysis of the results it seems desirable to present in brief form the significant protocols including the history of the patient, the essential pathological findings in the human nervous system, and the type of experimental disease produced in the monkeys inoculated with the various tissues. The inoculations resulting in the demonstration of poliomyelitis virus in certain tissues of the first seven cases are summarized in Table II.

Case 1 (Ohio).—Art., 5 year old white boy. Onset Aug. 12, 1940, with headache, vomiting, and fever (101°F.). Aug. 13, vomiting relieved; fever 101°F. Aug. 14, a.m., paralysis both arms, left leg, and face; p.m., paralysis left intercostal muscles, aphonia, and dysphagia; died 8:30 p.m. in respiratory failure.

Body not in refrigerator; necropsy begun 5½ hours after death. Only remarkable gross finding was extreme enlargement of cervical lymph nodes and extreme softness and mushrooming of spinal cord. Microscopically there was extensive neuronophagia and cuffing throughout the spinal cord, more marked on one side than on the other at the lumbar level, and with complete destruction of almost all the nerve cells in the thoracic and cervical levels. Similar focal lesions were present in the medulla but less marked than in the spinal cord.

Case 2 (Ohio).—Wil., 8 year old white boy. Reported not to have been feeling well and to have had some fever for 2 weeks before onset on Sept. 7, 1940, with abdominal pain, vomiting, and headache. Sept. 8, vomiting and fever. Sept. 9, nasal voice and dysphagia; on admission to hospital, temperature 102°, stiff neck, questionable right facial paralysis, palatal paralysis, hoarse nasal voice to no voice at all; no paralysis of the extremities or loss of deep tendon reflexes; pleocytosis of 500 cells, mostly lymphocytes. Sept. 10, temperature 103°, extremely restless and general condition worse; died 9:30 p.m.

Body in refrigerator; necropsy begun 8½ hours after death. The marked enlargement and edema in the gross of all the lymph nodes—cervical, mesenteric, axillary, and inguinal—and small white foci in parts of the liver were the remarkable findings. Microscopically, one level of the medulla presented heavy cuffing and extensive neuronophagia affecting chiefly the nucleus of the hypoglossal nerve on one side, and the nuclei ambigui and reticular substance of both sides. Despite the absence of obvious paralysis of the extremities and the presence of the reflexes, a section through the midcervical region of the spinal cord revealed neuronophagia of about half the number of the anterior horn cells and heavy cuffing, while sections through the thoracic and lumbar levels also showed focal neuronophagia and heavy cuffing. There was necrosis, slight polymorphonuclear cell infiltration, and proliferation affecting chiefly the reticulum of all the lymphoid tissue in the nodes, spleen, and intestines. The liver showed numerous areas of focal necrosis

TABLE II

Data on Tissues in Which Poliomyelitis Virus Was Demonstrated

Case No.	Tissue	Dose and route	Monkey No.	Result of inoculation				Passage
				Fever	Paralysis	Died or killed	Pathology	
		cc.						
1	Spinal cord	2 i.c. 15 i.p.	Rh 1-23	0	6* (A + L)	K 6*	Typical	+ (1/1)
	Medulla and pons	2 i.c. 27 i.p.	Rh 1-58	0	14 (Wk., 96.4°)	K 14	"	
	Diencephalon	2 i.c. 30 i.p.	Rh 1-54	7, 8*	8-12 (RF, L)	K 12	"	
	Motor cortex	2 i.c. 50 i.p.	Rh 1-56	7	8-10 (L)	K 10	"	
	Ileum—contents	2 i.c. 3.5 i.p.	Rh 1-50	0	5 (Aph., Prostr.)	D 6	"	+ (1/1) NP
	Ileum—washed wall	2 i.c. 45 i.p.	Rh 1-49	0	15-17 (LA)	K 17	"	0 (0/1)
	Pharyngeal + tonsils	2 i.c. (r15) 6 i.p.	Rh 1-46	0	0	K 39	NP	0 (0/1)
	Axillary + inguinal lymph nodes	2 i.c. (r16) 17 i.p.	Rh 1-40	irr.	0	K 36	NP	0 (0/2)
	Lung + liver + spleen + kidney**	2 i.c. (r16) 40 i.p.	Rh 1-39	irr.	0	K 36	NP	+ (1/2)
2	Spinal cord	2 i.c. 26 i.p.	Rh 1-91	0	11-12 (L)	K 12	Typical	+ (1/1)
	Medulla and pons	2 i.c. 20 i.p.	Rh 1-60	0	0	K 36	NP	
	Mesencephalon	2 i.c. 46 i.p.	Rh 2-16	8	10-11 (A)	K 11	Typical	0 (0/1)
	Diencephalon	2 i.c. 30 i.p.	Rh 2-17	0	13-17 (part. RA, LA)	K 17	"	
	Motor cortex	2 i.c. 44 i.p.	Rh 2-18	0	15-19 (trans. L)	K 31	"	
	Pharyngeal tissue	2 i.c. 17 i.p. 4 i.n.	Rh 2-24	0	11-13 (RF, N)	K 13	"	+ (1/1)

Legend.—A, both arms; L, both legs; RA, right arm; LA, left arm; RL, right leg; LL, left leg; F, facial; N, neck; Aph., aphonia; Prostr., prostrate; Wk., weakness; part., partial; trans., transitory paralysis; Rh, *rhesus;* Cyn, *cynomolgus;* NP, non-paralytic; r15, reinoculated on 15th day; i.c., intracerebral; i.p., intraabdominal; i.n., intranasal; irr., temperature irregular.

* Numerals refer to day of fever, paralysis, death, or sacrifice. D, dead; K, killed.

** *Case 1. Pooled Viscera.*—When it was established that the virus was contained in the pooled viscera, an attempt was made to discover in which it was present. The remaining pieces, all contained in one bottle of glycerol, were thoroughly washed in physiologic salt solution, and what was left of each organ was inoculated into separate *rhesus* monkeys as follows: *lung*—2 cc., i.c. and 5.5 cc., i.p. with reinoculation i.c. of 2 cc. 7 days later, *liver*—2 cc., i.c., 12 cc., i.p.; and reinjection i.c. 7 days later, *kidney*—2 cc., i.c., 18 cc., i.p.; and reinjection i.c. 7 days later. All monkeys remained well and no histologic evidence of poliomyelitis was found.

TABLE II—*Continued*

Case No.	Tissue	Dose and route	Monkey No.	Result of inoculation				Passage
				Fever	Paralysis	Died or killed	Pathology	
		cc.						
Case 2—*cont'd.*	Ileum—contents‡	2 i.c. 21 i.p. 12 i.n.	Rh 2-59	15, 16	17–20 (part. RA, L)	K 20	Typical	
	Descending colon—contents	20 i.n. 20 i.p. 6 i.p.	Rh 2-50	8, 9, 15, 16	18–19 (part. A + L)	K 19	Typical (olfactory +)	
3	Spinal cord (cervical)	2 i.c. 21 i.p.	Rh 1-97	8, 9	9–11 (F, Wk. N, part. RL)	K 11	Typical	+ (NP)
	Medulla and pons	2 i.c. 20 i.p.	Rh 1-27	0	13–15 (N, A, L, Prostr.)	K 15	"	
	Mesencephalon	2 i.c. 43 i.p.	Rh 1-85	7	10 (RL, part. LL)	K 10	"	
	Diencephalon	2 i.c. 25 i.p.	Rh 1-84	8	9–11 (RF, RL, part. LL)	K 11	"	
	Ileum—washed wall	2 i.c. 40 i.p.	Rh 1-78	0	0	K 35	0	
		2 i.c. 70 i.p.	Rh 2-55	20	21–22 (RL, part. LL)	K 22	Typical	+ (2/2)
	Descending colon—contents	12 i.p.	Rh 1-81	0	14–15 (RF, N, RL, part. LL + A)	K 15	Typical (olfactory—0)	+ (1/1)
4	Spinal cord	2 i.c. 10 i.p.	Rh 1-09	13, 14	0, Tr. 15, 16	K 21	Typical	0 (0/1)
	Medulla and pons	2 i.c. 10 i.p.	Rh 76	6–9	0, Wk. RL 13, 14	K 20	"	
	Motor cortex	2 i.c. 36 i.p.	Rh 1-12	0	0	K 37	NP	0 (0/1)
	Mesencephalon	2 i.c. (r15) 26 i.p. (r15)	Rh 1-10	0	0	K 37	NP	
	Descending colon—contents	22 i.p.	Rh 20	12, 13	14–16 (part. A + L)	K 16	Typical (olfactory—0)	+ (NP)
5	Spinal cord	2 i.c. 27 i.p.	Rh 1-93	0	0	K 35	0	
		2 i.c. 26 i.c.	Rh 2-40	0	9–16 trans.?	K 18	Typical	
		2 i.c. 26 i.p.	Cyn 2-39	0	15–19 (part. RL, Wk. L)	K 19	"	

‡ *Case 2. Ileum—Contents.*—8 gm. contents suspended in 100 cc. distilled water; etherized. 30 colonies per cc. after etherization. Rh 2-25—2 cc., i.c. and 1 cc. of untreated sediment in each nostril—dead following morning with bacterial meningitis. Rh 2-26—20 cc., i.p. on Oct. 13 and same on Oct. 15 with nasal instillations on 3 successive days—dead 6th day with acute peritonitis. Remaining material etherized second time and 0.1 cc. yielded no growth; inoculated into Rh 2-59.

TABLE II—*Concluded*

Case No.	Tissue	Dose and route	Monkey No.	Result of inoculation				Passage
				Fever	Paralysis	Died or killed	Pathology	
		cc.						
Case 5—*cont'd.*	Pharyngeal + tonsils	2 i.c. 35 i p.	Rh 3-04	11, 21, 23	0	K 35	NP	0 (0/2)
	Abdominal sympathetic plexus§	2 i.c.	Rh 3-14	irr.	0	K 35	Only in medulla	+ (1/2)
	Descending colon—contents	12 i.n. 20 i.p. 10 i.p.	Rh 3-21	irr.	20–24 (part. LL)	K 24	Typical (olfactory +)	
6	Spinal cord (cervical)	2 i.c. 21 i.p.	Rh 1-96	0	0	K 35	0	
	Various regions	2 i.c. 23 i.p.	Rh 2-43	irr.	0	K 36	NP	
		2 i.c. 23 i.p.	Cyn 2-44	9, 10	13–16(RL)	K 16	Typical	
	Mesencephalon	2 i.c. (r7) 32 i.p.	Rh 2-99	6, 9	9–11 (L, part. A)	K 11	"	
	Diencephalon	2 i.c. (r7) 28 i.p.	Cyn 2-97	6	9–10 (L)	K 10	"	+ (3/3)
	Pharyngeal + tonsils	2 i.c. 15 i.p.	Cyn 2-84	irr.	8–12 (L, RA, part. LA)	K 12	"	
	Ileum—washed wall	2 i.c. 36 i.p.	Cyn 2-88	irr.	11–12 (F, L, Wk. A)	K 12	"	+ (2/2)
	Descending colon—washed wall	2 i.c. 40 i.p.	Cyn 2-86	14	17–18 (A + L)	K 18	"	
	Descending colon—contents	2 i.c. 24 i.p. 6 i.n.	Rh 2-85	12, 13, 15, 16	17–18 (LL, part. RL + A)	K 18	Typical (olfactory +)	
7	Motor cortex	2 i.c. (r7) 43 i.p.	Rh 3-33	12, 13, 14	14–17 (part. LL, Wk. RL)	K 17	Typical	
	Descending colon—contents	20 i.p. 114 i.n. 6 i.p.	Rh 2-01	0	12–13 (RL, Aph., part. LL + A)	K 13	Typical (olfactory—0)	

§ *Case 5. Abdominal Sympathetic Plexus.*—Histological examination of the nervous system of Rh 3-14 did not satisfy the criteria for a diagnosis of non-paralytic poliomyelitis because in none of the six levels of the spinal cord was there any sign of outfall of neurons, interstitial infiltration, or degenerative reaction in the roots. There was, however, cuffing of several vessels in the substance of the medulla associated with interstitial glial infiltration in the reticular substance of one side and in the region of one of the vagal nuclei on the other. Consequently, a piece of the medulla which had been saved in 50 per cent glycerol in the refrigerator, was passaged to two *rhesus* monkeys. One of these developed typical poliomyelitis on the 14th day with complete flaccid paralysis of both legs, and partial paralysis of the arms; it was sacrificed on the 15th day when the temperature dropped from 104.8–101°. Microscopically there was extensive neuronal necrosis, neuronophagia, and cuffing in the spinal cord and medulla. Passage of material from this monkey again produced paralytic poliomyelitis in another monkey, and the virus was not pathogenic for mice.

with marked proliferation of mononuclear cells and epitheloid cell elements, the latter fusing to form small giant cells. Examination of Meissner's and Auerbach's plexuses in the ileum suggested that a number of these nerve cell collections, especially those of the myenteric plexus which were more readily discerned, had undergone necrosis.

Case 3 (Indiana).—Hoo., 16 year old white boy. Onset Aug. 13, 1940, with fever, sore throat, headache. Aug., 14, same. Aug. 15, up and about during the day and attended fair; at night fever, stiff neck, and headache. Aug. 16, same and vomiting. Aug. 17, dysphagia, on admission to hospital, temperature 104°, stiff back, dysphagia, and pleocytosis of 27 cells; became very restless, vomited, and developed difficulty in respiration. Aug. 18, fibrillary twitching noted over face and chest; died 6:30 a.m.

Body in refrigerator; necropsy begun 8 hours after death. In the gross, there was marked enlargement of the cervical, mesenteric, axillary, and inguinal lymph nodes. Microscopically one level of the medulla showed extensive neuronophagia and cuffing, affecting chiefly the nuclei ambigui and other nuclei of 9th and 10th cranial nerves, the hypoglossal nuclei, and the reticular substance; there was also extensive neuronophagia and cuffiing in a midcervical section of the spinal cord, focal neuronophagia and cuffing in a midthoracic section, but no changes whatever in a midlumbar region. A section of the abdominal sympathetic plexus revealed two questionable minute foci of interstitial and perivascular infiltration with mononuclear cells in one of the ganglia.

Case 4 (Indiana).—Wen., 13 year old white boy. Onset July 27, 1940, with sore throat. July 22, p.m., vomited, speech difficulty (nasal quality and poor enunciation) and dysphagia. July 29, temperature 103°, walked into hospital; obviously ill, very restless, stiff neck, dysphagia, dysphonia, and very questionable facial and hypoglossal nerve involvement; reflexes present though hypoactive; pleocytosis of 80 cells. July 30, 4 a.m., 103.6°; secretions accumulating in back of throat, vomited, had tonic convulsion and opisthotonos terminally; died about 6 a.m.

Body refrigerated until necropsy was begun 8 hours after death. Microscopically there was extensive necrosis, neuronophagia and cuffing affecting most of one side and part of the other side of a midcervical section of the spinal cord, and focal neuronophagia and cuffing in the midthoracic and midlumbar levels; one section of medulla at level of decussation of pyramids showed only diffuse cuffing.

Case 5 (West Virginia).—Bon., 16 year old white boy. Onset Aug. 21, 1940, with headache and stiffness of neck coming on in the evening after he played baseball all day. Aug. 22, left work and walked into hospital because someone frightened him by saying that he might have "polio;" temperature 99.4°, neck and back stiff and painful, reflexes variable, some hyperactive and others elicited with difficulty; cerebrospinal fluid clear, under normal pressure, qualitative test for globulin negative, and 20 white cells (mostly polymorphonuclear) per c. mm. Aug. 23, marked dysphagia and dyspnea but no paralysis of extremities. Aug. 24, hoarse speech and crowing respiration with laryngeal airway patent; placed in respirator and 350 cc. of convalescent blood given intravenously. Aug. 25, dysarthria, dysphagia, and dyspnea increased in severity; died 3:30 a.m.

Body at room temperature; necropsy begun 8 hours after death. Microscopically there was extensive neuronal destruction, cellular infiltration, and cuffing affecting

chiefly both nuclei ambigui and the reticular substance in one section of the medulla. One section through the midcervical spinal cord showed extensive destruction of neurons, neuronophagia and cuffing with few nerve cells left intact; the midthoracic and midlumbar sections revealed many foci of neuronophagia and heavy cuffing but most nerve cells were preserved.

Case 6 (West Virginia).—Pers., 3 year old white girl. Onset Aug. 7, 1940, with sore throat and "some fever." Aug. 10, complete left facial paralysis, some stiffness of neck and back, and 165 white cells per c.mm. of cerebrospinal fluid. Aug. 11, dysphagia, generalized weakness of extremities, and somewhat stuporous. Aug. 13, comatose in a.m.; temperature 106°, and 90 cells per c.mm. of cerebrospinal fluid; died 4:30 p.m.

Necropsy begun 2½ hours after death. Poliomyelitis lesions in medulla and spinal cord. Since the first inoculation of the spinal cord and medulla into *rhesus* monkeys yielded negative results, it was decided to inoculate the remaining tissues into as many *cynomolgus* monkeys as could be obtained at the time.

Case 7 (Ohio).—Roo., 15 year old white girl. Onset Aug. 21, 1940, with headache and backache. Aug. 22, anorexia, vomiting, constipation, stiff neck and back. Aug. 23, paralysis of the upper and lower extremities, and difficulty in breathing requiring artificial respiration; on admission to the hospital some movement was found in the left arm and leg but the respiratory excursion was nil; temperature 102.5–104°. Aug. 24, temperature 101.8–104°; complete paralysis of both upper and lower extremities, weakness of muscles supplied by the left 5th and 12th cranial nerves. Aug. 25 and 26, temperature 102.6–102.8°, pulse 120 to 145, periodic cyanosis, dysphagia, incontinent of urine and feces. Aug. 27, died 1:45 a.m.

Body refrigerated until necropsy was begun 10 hours after death. Microscopically all levels of the spinal cord showed the most extensive destruction of neurons, neuronophagia, interstitial infiltration, cuffing, and meningeal infiltration; in the lumbar and thoracic levels an occasional nerve cell was still present, while in the cervical region the destruction appeared to be complete. The single level of the medulla examined exhibited heavy cuffing and focal neuronophagia which was especially concentrated on one side in an area corresponding to the nucleus solitarius.

This case was remarkable for the fact that no virus was demonstrated in the spinal cord despite the fact that 3 monkeys—2 *rhesus* and 1 *cynomolgus*—were inoculated with large amounts of the tissue. The isolation of virus from the motor cortex and the colon contents suggests that one is not dealing with a special strain of virus of low pathogenicity for monkeys but rather that there may perhaps be relatively rapid destruction of the virus where the inflammatory and phagocytic reaction is especially marked.

Case 8 (Indiana).—Hun., 6 year old white boy. Aug. 26, 1940, headache, given castor oil for constipation. Aug. 27, 28, 29, apparently well; soaked in rain on Aug. 28. Aug. 30, headache; examination by local physician revealed only "bowel trouble." Sept. 1, vomiting and "spitting of foamy material," dysphagia, and stiff and painful neck. Sept. 2, admitted to hospital; unable to swallow or talk well, neck rigid and Kernig sign positive, all reflexes present, 14 white cells per c.mm. of cerebrospinal fluid, temperature 104.6–105.6°. Sept. 3, became cyanotic; in respirator; died 10:45 a.m.

Body refrigerated until necropsy which was begun 7 hours after death. The only

remarkable findings in the gross were that the tonsils were necrotic and "cheesy" and the cervical lymph nodes were more markedly enlarged than any other group. Microscopically, there was neuronophagia of many anterior horn cells and cuffing in the midlumbar section, focal neuronophagia in the midthoracic, and neuronophagia of most anterior horn cells on one side and of many on the other side, as well as cuffing and meningeal infiltration in the midcervical section of the spinal cord. The single section of the medulla revealed heavy cuffing and focal neuronophagia which was especially concentrated in the dorsal motor nucleus of the vagus.

Search for Virus.—Despite the unquestionable typical lesions, the short duration of the paralysis, preservation of the tissues in the frozen state immediately after their removal from the body, inoculation of the spinal cord into 3 monkeys (1 *cynomolgus*, and 2 *rhesus*), of the medulla and pons, and both olfactory bulbs into one monkey each, did not yield any evidence of the presence of virus. Mice inoculated with the human medulla and pons suspension remained well. While all the other tissues listed in Table I were obtained in this case, they were not tested. The descending colon was free of contents.

Case 9 (Indiana).—Rip., 21 year old, white, female nurse who became ill 3 days after she started to take care of a patient with poliomyelitis. Onset Aug. 8, 1940, fainting attack, sore throat. Aug. 9, ached all over, dizzy, sore throat. Aug. 10, nausea, "rhinitis." Aug. 11, difficulty in talking, nausea, neckache, and pain between the shoulders. Aug. 12, rigidity of neck and back; local physician found paralysis of the throat and 130 white cells (70 per cent polymorphonuclear) per c.mm. of cerebrospinal fluid. Aug. 13, weakness of right shoulder and irregular, shallow respiration, 360 cc. of convalescent serum administered. Aug. 14, admitted to hospital and placed in respirator; unable to swallow, talked poorly, cyanotic. On Aug. 18, left arm weak; Aug. 20, became irrational; Aug. 22, "the heart beat became rapid," respirations short, rapid, and not in rhythm with the respirator. Aug. 23, cyanosis and collapse with pulse slowed to 50; died 9:40 a.m. Fever present throughout and varied from 100–103.6°.

Body refrigerated until the necropsy was begun 5½ hours after death. Microscopically, one section of the medulla revealed a symmetrical necrotic lesion (older than any of the others) at the site of both nuclei ambigui, in addition to heavy cuffing and more recent foci of neuronophagia. In the midcervical section of the spinal cord there was practically complete destruction of the anterior horn cells of one side with less involvement on the other side; the anterior horns were also markedly affected in the thoracic section, and while distinct neuronal lesions were present in the lumbar cord more cells were spared here than in the other levels.

Search for Virus.—This case was especially included in this series to determine whether or not the spread of virus is more diffuse when the disease had lasted longer. However, since no virus was demonstrated in the spinal cord which was inoculated into 3 monkeys (1 *cynomolgus* and 2 *rhesus*), in the medulla and pons, and the olfactory bulbs, the remaining tissues were not investigated. 10 young mice, inoculated with the medulla and pons suspension, all remained well. The colon contents which weighed only 1.4 gm. were suspended in 20 cc. of distilled H_2O; 15 cc. of the etherized centrifuged preparation was injected intraabdominally and 1 cc. of the resuspended untreated sediment was instilled intranasally on two successive days into Rh 3-50. The monkey remained well and histological examination revealed no poliomyelitis lesions.

Case 10 (Illinois).—The necropsy on this case was performed by Dr. Victor Levine of Chicago who sent us both olfactory bulbs and part of the cervical spinal cord in 50 per cent glycerol in separate bottles as well as the history of the patient and the histological sections.

The patient was a 22 year old white woman. Onset Sept. 10, 1940, with headache, pain in the back and upper thighs, general malaise, anorexia, and occasional nausea and vomiting. Paralysis developed Sept. 11, and on admission to the hospital on Sept. 13 she showed almost complete paralysis of both legs and of the abdominal muscles, poor excursion of the lower ribs, marked weakness of both arms, head drop, and stiff neck; temperature 101.4°, and the cerebrospinal fluid contained 250 white cells per c.mm. On Sept. 13 and 14 she received 380 cc. of convalescent serum. Respiratory difficulty, cyanosis, and tremors of the arms appeared early Sept. 15 and she was placed in a respirator. Paralysis finally involved the diaphragm, intercostals, upper extremities, and muscles of deglutition, and the patient died on Sept. 16. The necropsy was performed 2½ hours after death. Microscopically there was complete destruction of all nerve cells in the lumbar and thoracic sections and of practically all nerve cells in the cervical sections of the spinal cord, with extensive cuffing, interstitial infiltration and neuronophagia. Similar lesions, though less extensive, were present in the medulla.

Tissues Tested for Virus

Spinal Cord.—2 cc. i.c. and 11 cc. i.p. into Rh 2-37. Temperature below 103.5° for first 5 days, 105.5° with excitement and weakness of left hand 6th day, 103.8° with complete paralysis of left upper extremity on 7th day, 101.7° and monkey almost prostrate on 8th day when it was sacrificed. Typical severe poliomyelitis lesions.

Olfactory Bulbs.—Both bulbs ground without an abrasive, suspended in 2 cc. distilled water, and injected i.c. into Rh 2-03. Remained entirely well, sacrificed 35th day, and no poliomyelitis lesions were found.

Case 11 (Indiana).—Dau., 2 year and 10 months old white boy. Onset Aug. 9, 1940, with vomiting and "fever." Anorexia, restlessness and "fever" persisted; paralysis appeared Aug. 13 and on admission to the hospital he exhibited left facial paralysis, nystagmus of the right eye, "gurgling in throat," rigidity of neck and back, and convulsive movements; motions and reflexes present in both arms and legs; 28 white cells per c.mm. of cerebrospinal fluid; temperature 104–106.2°. Died Aug. 14.

Body refrigerated but necropsy not done until 24 hours after death. Only few tissues obtained. Microscopically there was extensive necrosis of nerve cells, neuronophagia, and cuffing in the medulla, while in the two available sections of the spinal cord, *i.e.* lower cervical and thoracic, there was chiefly cuffing and almost all cells were preserved.

Tissues Tested for Virus

Spinal Cord and Medulla.—2 cc. i.c. and 15 cc. i.p. into Rh 1-25. Remained well, sacrificed 36th day, and no poliomyelitis lesions found. 10 young mice inoculated with human material remained well.

Olfactory Bulbs.—Both bulbs ground without abrasive suspended in 1.5 cc. physiologic salt solution and injected i.c. into Rh 1-24. Remained well, sacrificed 36th day, and no poliomyelitis lesions found.

Contents of Descending Colon.—This material was frozen for 4 months before it was

tested. 20 per cent suspension was used and Rh 3-49 received 21 cc. i.p. the first day, followed by another i.p. injection of 20 cc. in 24 hours, and daily instillations of 1 cc. of untreated suspension into each nostril for 10 days. No fever; partial paralysis of both lower extremities 13th day, and complete paralysis of left lower and partial paralysis of all the other extremities 14th day, when the monkey was sacrificed. Typical severe poliomyelitis lesions were found in the spinal cord and medulla, and their presence in one olfactory bulb and on one side of the anterior perforated substance suggests that the virus invaded by the olfactory pathway.

Summary and Analysis of Data

Table III gives some of the pertinent data on the nine complete and two partial poliomyelitis necropsies and shows that while the presence of virus

TABLE III

Human Poliomyelitis Necropsies

Search for Virus in Olfactory Bulbs

Patient	Age	Time since onset of		Primary paralysis	Tests for virus in	
		First symptoms	Paralysis		Olfactory bulbs	Other nervous tissues
	yrs.	*days*	*days*			
1. Art.	5	2½	½	U, L, Fac. (terminal bulbar)	0	+
2. Wil.	8	3	1	Bulbar	0	+
3. Hoo.	16	5	1	"	0	+
4. Wen.	13	3	2	"	—	+
5. Bon.	16	4	2	"	0	+
6. Per.	3	6	3	"	0	+
7. Roo.	15	6	4	U, L, Intercost. (terminal bulbar)	0	+
8. Hun.	6	4 (8?)	2	Bulbar	0	0
9. Rip.	21	15	12	"	0	0
10. PM 56 (partial)	22	6	5	U, L, Intercost. (terminal bulbar)	0	+
11. Dau. (partial)	2½	5	1	Bulbar	0	0, (+)*

U = upper extremities; L = lower extremities; Fac. = facial.

* Virus present in contents of descending colon.

was in no instance demonstrated in the olfactory bulbs, it was found elsewhere in the body in all but two cases. It is noteworthy and significant that three of the cases which contained demonstrable virus in the nervous system but not in the olfactory bulbs, began with paralysis of the extremities and were not primarily of the bulbar type.

The results of tests for virus in seven cases of poliomyelitis in which all the selected tissues were studied are presented in Table IV. It is apparent that partly due to the difficulty inherent in the transmission of human virus to monkeys and perhaps also because of the longer duration of the illness in some instances, the virus was not consistently demonstrated in any one tissue. However, when the tests on all the cases are pooled a distinct pattern of virus distribution emerges in which certain groups of tissues

yield positive results with considerable regularity while others are consistently negative. Thus, in the central nervous system, the olfactory bulbs, the anterior perforated substance with the adjacent corpus striatum, and

TABLE IV

Distribution of Virus in Human Poliomyelitis

Tissues tested	Case No., type and duration of illness						
	1 Spino-bulbar S 2.5 d.* Par. 0.5 d.	2 Bulbar S 3 d. Par. 1 d.	3 Bulbar S 5 d. Par. 1 d.	4 Bulbar S 3 d. Par. 2 d.	5 Bulbar S 4 d. Par. 2 d.	6 Bulbar S 6 d. Par. 3 d.	7 Spino-bulbar S 6 d. Par. 4 d.
1. Olfactory bulbs	0	0	0	—	0	0	0
2. Ant. perf. substance, etc.	0	0	0	0	0	0	0
3. Ant. front. + occipit. cortex	0	0	0	0	0	0	0
4. Motor cortex	P	P	0	NP	0	0	P
5. Diencephalon	P	P	P	0	0	P^c (+)	0
6. Mesencephalon	0	P	P	NP	0	P	0
7. Medulla (+ pons)	P	NP	P	NP	0	0	0
8. Spinal cord	P (+)	P (+)	P (+)	NP	0, P, P^c	0, NP, P^c	0, 0, 0^c
9. Sup. cerv. sympathetic ganglia	0	0	—	0	0	0^c	0
10. Abdominal " "	0	0	0	0	NP (+)	0^c	0
11. Suprarenals	0	0	0	0	0	0^c	0^c
12. Salivary glands	0	0	0 (10 d.)**	0	0	0^c	0^c
13. Cervical lymph nodes	0	0	0	0	0	0^c	0^c
14. Mesenteric " "	0	0	0	0	0	0^c	—
15. Axill. + inguin. lymph nodes	NP	0	0	0	0	0^c	0^c
16. Lungs + liver + spleen + kidneys	NP (+)	0	0	0	0	0^c	0^c
17. Nasal mucosa	0	0	0	0 (15 d.)	0	0^c	0
18. Pharyng. mucosa ± tonsils	NP	P (+)‡	0	0	NP	P^c	0
19. Ileum—washed wall	P	0	0, P (+)	0, 0	0	P^c (+)	0
20. " —contents	P (+)	P	0	0	0	0	0
21. Desc. colon—washed wall	0	0	0	0	0	P^c	0
22. " " —contents	Empty	P	P (+)	P (+)	P	P	P

P, paralytic poliomyelitis in inoculated monkey.
NP, non-paralytic " " " "
0, no evidence of poliomyelitis.
c, indicates that a *cynomolgus* monkey was used for the test.
(+), passage positive.
* S 2.5 d., total duration of illness was 2.5 days.
Par. 1 d., paralysis 1 day.
** (10 d.), monkey died on 10th day.
‡ No tonsils in this case.

the anterior frontal and occipital portions of the neopallial cortex were consistently negative, while the motor cortex, diencephalon, mesencephalon, medulla and pons, and spinal cord were predominantly positive. This distribution of virus in the central nervous system is in good agreement not only with the known distribution of neuronal lesions but also with a progression of virus along specific pathways and insulated tracts. Next to

these areas in the central nervous system, the virus was predominantly situated in the alimentary tract. The pharyngeal mucosa alone or together with the tonsils was positive in four of the seven cases, and it is our belief that the many early positive findings with human tonsils are probably indicative of the presence of virus in the attached pharyngeal mucosa rather than in the tonsils themselves. This belief is based not only on the presence of the virus in the pharyngeal mucosa in the absence of tonsils, but also on the consistently negative results with the cervical lymph nodes in this series. When one recalls the fact that only a small portion of the entire small intestine was being tested, it is indeed remarkable that the washed wall of the ileum was positive in three cases and the contents in two. Very significant also is the finding that the contents of the descending colon contained virus in each one of the six cases in which contents were available, while the washed wall was positive only once. The possibility that the presence of virus in the washed intestinal wall might be due to incomplete washing away of the contents was, of course, considered and found to be especially remote as regards the wall of the ileum since the washed wall was positive on two occasions when the total contents of the entire segment were negative. In the descending colon, however, the reverse was true and the almost regular presence of virus in the contents suggests that virus originating elsewhere in the alimentary tract is concentrated in the colon.

The regularity with which the virus was demonstrated in the contents of the descending colon is not readily accounted for by the methods used, since we did not obtain the same results with the stools of living patients. It would appear either that patients in whom the disease terminates fatally have more virus in their intestinal tract, or, and it seems rather unlikely, that something happens to the virus between the descending colon and the rectum.

In the next group of tissues one finds that the nasal mucosa, the salivary glands, the superior cervical sympathetic ganglia, the suprarenals, and the cervical and mesenteric lymph nodes were consistently negative whether tested in *rhesus* or *cynomolgus* monkeys. This finding suggests a number of points which are of significance in understanding the nature of human poliomyelitis: (*a*) that the previously reported finding of virus in the nasal mucosa in a single case of human poliomyelitis represents a result that is either rare and fortuitous or due to contamination; (*b*) that the centrifugal spread of virus, which is so common in rabies, does not appear to occur in human poliomyelitis; (*c*) the negative results with the salivary glands suggest that the virus is not likely to be eliminated by way of the saliva; (*d*) the negative results with the superior cervical sympathetic ganglia are an

indication not only of the absence of any appreciable centrifugal spread but also that the virus did not spread centripetally along this sympathetic pathway; (*e*) that virus which is demonstrable in the wall of the pharynx or ileum is probably there because that is its portal of entry and not because it has become established there in a centrifugal spread of the virus, for if the latter were true it should also have been present in the suprarenals, salivary glands, etc.; (*f*) that virus which is present in the contents and wall of the alimentary tract is not readily absorbed or is at least not demonstrable in the lymph nodes draining those areas despite the fact that they exhibit distinct pathologic changes; these results with the lymph nodes are in agreement with those of numerous tests by Flexner (3), and the more recent report of Kling, Olin, and Gard (4) in which the virus was presumably demonstrated on rare occasions in the mesenteric (4 of 33 specimens) and cervical (1 of 25 specimens) lymph nodes may perhaps be accounted for partly on the basis that their criteria for experimental poliomyelitis are in some cases not acceptable (5) and partly that the virus may actually get get into these lymph nodes on rare occasions. It is of interest in this respect that in mice which normally carry the virus of mouse polioencephalitis in their intestinal tract, Olitsky (12) found that the infective agent although present on occasion is not readily demonstrable in the mesenteric lymph nodes.

The presence of virus in the pool of axillary and inguinal lymph nodes and in the pool of the lungs, liver, spleen, and kidneys in the first case in which the patient died after an illness of only 2½ days, at first suggested that the virus may perhaps be more widely distributed in the early stages of the disease. Until further data become available, however, the following alternative interpretation appeals to us more: (*a*) that the pool of lymph nodes was positive not because of the axillary nodes but because of the inguinals which drain the perianal region where virus from the intestinal contents might enter through a fissure, and (*b*) that the lungs containing aspirated material from the alimentary tract probably contributed the virus to the pool of the viscera.

Although the abdominal sympathetic plexus was tested in all seven cases, five of which were of the bulbar type and two spino-bulbar, evidence of its presence was obtained in only one instance and that in a bulbar case. While the monkey inoculated with the human material developed neither paralysis nor lesions in the spinal cord and exhibited only interstitial infiltration and perivascular cuffing in the medulla, the production of the typical paralytic disease on passage can leave little doubt of the presence of virus in the abdominal sympathetic plexus of this case. It will be necessary to obtain

additional data with the abdominal sympathetic ganglia (dissected with especial care to include its superior mesenteric component—and we cannot be certain that we always had this component in the present study) and especially from cases with ascending paralysis, which rarely terminate fatally, before much can be said about the significance of this finding. However, it is hardly necessary to stress that the presence of virus in the abdominal sympathetic plexus, under conditions pointing against generalized centrifugal spread, is in good agreement with progression of the virus from the lower portion of the alimentary tract along the sympathetic pathway. Should further investigations confirm the suggestion established by the present studies that human poliomyelitis is a disease in which the alimentary tract is primarily attacked with secondary involvement of the central nervous system, it is clear that, in the many instances in which only the lower extremities are affected, progression of the virus along the sympathetic fibers of the intestines through the abdominal sympathetic plexus is definitely to be expected. It is noteworthy, however, that even in the strictly bulbar type of the disease, the virus may be present not only in the walls of the pharynx but also in the ileum and colon.

DISCUSSION

Ever since human poliomyelitis was shown to be an infectious disease, various concepts of its essential nature have been entertained. Thus, it has been variously regarded (*a*) as a disease in which the virus multiplies in the upper respiratory tract and from there both invades the nervous system and escapes to infect others; (*b*) as a disease in which peripheral multiplication of the virus and the site from which the nervous system is invaded is limited to the olfactory portion of the nasal mucosa; (*c*) that the virus may perhaps enter by way of the skin either by means of an insect bite or direct contamination; and (*d*) as primarily a gastro-enteric infection partly because of its occurrence chiefly in the late summer and early autumn and the finding of virus in the stools, and partly because of the results of certain animal experiments. The data upon which the various hypotheses were based originated only in small part from direct studies on the human disease, and were derived mostly from observations on the behavior of the virus chiefly in *rhesus* monkeys and from certain epidemiological observations which could be interpreted in a number of different ways.

The upper respiratory tract hypothesis was based chiefly on the demonstration that virus could occasionally be isolated from nasopharyngeal washings (note the pharyngeal component), that it was present in the human tonsils (there was always attached pharyngeal mucosa), that it was pre-

sumably isolated in a single instance from the human nasal mucosa, and on the fact that next to intraneural injection, infection of the *rhesus* monkey was most readily accomplished by nasal instillation of the virus. When it was found in recent years that virus instilled intranasally in *rhesus* monkeys could invade the nervous system only by the olfactory pathway, the belief became almost general that the same was true of human poliomyelitis. It was then shown that distinct lesions were regularly present in the olfactory bulbs of monkeys when the nervous system was invaded by the olfactory pathway but not when the virus entered in any other way (9). These lesions which were thus indicators of the olfactory portal of entry were not found, however, in the human olfactory bulbs despite the fact that thousands of sections were studied (10). The skin as the site of primary attack was suggested by the presumably greater infectivity by the intracutaneous route of some freshly isolated strains (13), and by the occurrence of poliomyelitis following subcutaneous injection of certain vaccines in human beings (14). This hypothesis was obviously incomplete in explaining poliomyelitis as an epidemic disease which is presumably limited to man. The gastro-enteric hypothesis has been through many vicissitudes. In 1912, Kling, Wernstedt, and Pettersson (15) obtained the virus (proved by positive passage in at least one case) from the washings of the small intestine in three of six fatal cases of human poliomyelitis, but they also reported that in the same series of cases the virus was present in the combined washings from the mouth and nose, as well as in the washings of the trachea. This did not elucidate the origin of the virus and its presence in the washings of the small intestine was usually interpreted as representing virus that had been swallowed. However, the clues contained in the work of Kling, Wernstedt, and Pettersson were not pursued and remained dormant for a quarter of a century chiefly because in their subsequent work on intestinal contents and nasopharyngeal washings from patients and contacts it became apparent that they often employed criteria for the presence of virus which were later found not to be acceptable. The whole work was set aside without attempting to extract the undeniably significant and valid results. In the past few years, however, with the development of more adequate methods, incontrovertible evidence, has been brought forth for the presence of virus in the stools of patients with paralytic or non-paralytic poliomyelitis and in contacts as well (16). This important finding, however, threw little light on the essential nature of the human disease since there was as yet no indication that the virus in the stools did not have its origin in swallowed secretions. Experimental work on monkeys has until recently contributed little or nothing in support of the gastro-enteric hypothesis. The work on

rhesus monkeys in which poliomyelitis was produced by injecting large amounts of virus into the gut (17) was not accepted partly because it was not readily reproduced (3, 18) and partly because it did not approximate natural conditions. The results of earlier feeding experiments on *cynomolgus* monkeys were irregular and also inconclusive because there was no evidence that infection under those conditions had not occurred by the olfactory pathway. The more recent experiments of Burnet, Jackson, and Robertson (1) indicated that *cynomolgus* monkeys could probably be infected by the oral route without involving the olfactory pathway, and Howe and Bodian (2) established that chimpanzees, whose olfactory tracts had been severed, could develop poliomyelitis after being fed human stools containing the virus.

The results of the present investigation on the distribution of virus in the tissues of fatal cases of human poliomyelitis permit a revaluation of the various hypotheses in the light of the following findings in the human disease.

1. The absence of demonstrable virus in the olfactory bulbs and anterior perforated substance indicates that the olfactory pathway need not be affected in human beings.

2. The absence of infective virus in the nasal mucosa suggests that it is not the site of virus multiplication and dissemination.

3. The absence of virus in the salivary glands indicates that the saliva is not a likely means for its elimination.

4. The positive results with the tonsils and pharyngeal mucosa, are probably due to the pharyngeal tissue rather than the tonsils.

5. Next to the central nervous system the virus is distributed predominantly in the alimentary tract and is present not only in the contents but also in the washed walls of various parts of the tract including the pharynx, ileum, and occasionally the colon.

6. Infection of the walls of the alimentary tract appears to be the result neither of generalized dissemination of the virus nor of secondary centrifugal spread, but rather that of primary localization or portal of entry.

7. The distribution of virus in the central nervous system is limited to certain areas and is not as indiscriminately disseminated as viruses (*e.g.*, equine encephalomyelitis) which can invade through the blood vessels or those (*e.g.*, rabies), which having entered by a specific nervous pathway, are capable of extensive centrifugal spread.

8. In the absence of evidence of any appreciable centrifugal spread to peripheral collections of nerve cells, the demonstration of virus in the abdominal sympathetic ganglia of one case is significant in suggesting one of the possible routes of virus progression in certain instances.

The pattern of virus distribution in human poliomyelitis, as it emerges from the present study, thus points to almost the entire alimentary tract as the primary site of attack by the virus, and contains no support for the concepts involving either the olfactory pathway or the respiratory tract. This pattern of virus distribution also militates against the cutaneous route although it is conceivable that occasional infection by direct contamination of the broken skin may be possible. It is of interest to note how much similarity there appears to be between this picture of human poliomyelitis and that of Theiler's spontaneous mouse encephalomyelitis or poliomyelitis, in which Olitsky (19) first demonstrated that the virus is carried in the intestinal tract of many normal mice, although only 1 of 2000 to 5000 develops paralysis. The subsequent demonstration by Theiler and Gard (20) and by Olitsky (12) that the infectious agent is also present in the washed intestinal wall and that this is the most probable origin of the virus in the intestinal contents, brings out a most remarkable analogy between the two diseases.

CONCLUSIONS

1. Studies on a large number of tissues obtained from fatal cases of human poliomyelitis have revealed that the virus is distributed predominantly in two systems: (*a*) certain regions of the nervous system, and (*b*) the alimentary tract.

2. Poliomyelitis virus was demonstrated in the walls of the pharynx, ileum, and only once in those of the descending colon, while the contents of the descending colon regularly contained the virus.

3. The presence of virus in the walls of the alimentary tract appears to be the result neither of generalized dissemination of the virus nor of secondary centrifugal spread, but rather that of primary localization or portal of entry.

4. In the absence of evidence of any demonstrable centrifugal spread to peripheral collections of nerve cells (*e.g.*, in the superior cervical sympathetic ganglia, suprarenals, salivary glands), the presence of virus in the abdominal sympathetic plexus of one case may be indicative of at least one pathway of centripetal virus progression.

5. The absence of demonstrable virus in the nasal mucosa, olfactory bulbs, and anterior perforated substance suggests that neither the upper respiratory tract nor the olfactory pathway were affected in the cases of human poliomyelitis studied in the present investigation.

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